

IN THE CLAIMS

1. (previously presented) A modified cytochrome P450 monooxygenase which, in comparison with the wild-type enzyme, shows an altered substrate profile in the terminal and/or subterminal enzymatic hydroxylation of aliphatic carboxylic acids, owing to site-specific mutagenesis of its substrate binding region.
2. (previously presented) A monooxygenase as claimed in claim 1, which is derived from cytochrome P450 monooxygenases of bacterial origin.
3. (previously presented) A monooxygenase as claimed in claim 2, which is derived from *Bacillus megaterium* cytochrome P450 monooxygenase BM-3 with an amino acid sequence in accordance with SEQ ID NO: 2, which has at least one functional mutation in one of the following amino acid sequence regions: 24-28, 45-51, 70-72, 73-82, 86-88, 172-224 and 352-356, with the proviso that , if the enzyme carries the mutation F87A, more than one of these regions is mutated.
4. (previously presented) A monooxygenase as claimed in claim 3, which comprises at least one functional mutation in the amino acid sequence regions 86-88 and 172-224.
5. (previously presented) A monooxygenase as claimed in claim 4, which comprises at least one of the following amino acid substitution patterns:
 - a) F87V;
 - b) F87A L188K;
 - c) F87V L188K;
 - d) F87A L188K A74G;

- e) F87V L188K A74G;
- f) F87A L188K A74G R47F;
- g) F87V L188K A74G R47F;
- h) F87A L188K A74G R47F V26T; or
- i) F87V L188K A74G R47F V26T;

and functional equivalents thereof.

6. (previously presented) A monooxygenase as claimed in claim 3, which comprises a single amino acid substitution from amongst the following:

- a) V26T,
- b) R47F,
- c) S72G,
- d) A74G,
- e) F87V,
- f) L188z, where Z is an amino acid selected from amongst K, R, W, Q, N, G, A and S, and
- g) M354T;

and functional equivalents thereof.

7. (previously presented) A nucleic acid sequence encoding a monooxygenase as claimed in claim 1 and the complementary nucleic acid sequence thereof.

8. (previously presented) An expression construct comprising, under the genetic control of regulatory acid sequence, an encoding sequence which encompasses a nucleic acid sequence as claimed in claim 7.
9. (previously presented) A vector which encompasses at least one expression construct as claimed in claim 8.
10. (previously presented) A recombinant microorganism which has been transformed with at least one vector as claimed in claim 9.
11. (previously presented) A microorganism as claimed in claim 10, selected from amongst bacteria of the genus *Escherichia*.
12. (currently amended) A process for the enzymatic production of ~~terminally or~~ subterminally hydroxylated aliphatic carboxylic acids, which comprises
 - a1) culturing a recombinant microorganism which has been transformed with a vector which encompasses an expression construct comprising, under the genetic control of regulatory nucleic acid sequences, a sequence which encompasses a nucleic acid sequence encoding ~~the monooxygenase of claim 4~~ a modified cytochrome P450 monooxygenase which, in

comparison with the wild-type enzyme, shows an altered substrate profile in the subterminal enzymatic hydroxylation of aliphatic carboxylic acids, owing to site-specific mutagenesis of its substrate binding region, in the presence of a culture medium which contains at least one hydroxylatable C₈-C₁₂-carboxylic acid or at least one hydroxylatable C₈-C₁₂-carboxylic acid derivative; or

- a2) incubating a reaction medium containing at least one hydroxylatable C₈-C₁₂-carboxylic acid or at least one hydroxylatable C₈-C₁₂-carboxylic acid derivative with ~~an enzyme as claimed in claim 1~~ said monooxygenase, and
- b) isolating the resulting hydroxylated product from the medium.

13. (canceled)

14. (currently amended) A method as claimed in ~~claim 13~~ claim 12, wherein the hydroxylatable carboxylic acid is a C₈-C₁₂-monocarboxylic acid or a derivative thereof and the monooxygenase (SEQ ID NO: 2) used comprises at least one of the following amino acid substitution patterns:

- a) F87V;
- b) F87A L188K;
- c) F87V L188K;
- d) F87A L188K A74G;

- e) F87V L188K A74G;
 - f) F87A L188K A74G R47F;
 - g) F87V L188K A74G R47F;
 - h) F87A L188K A74G R47F V26T; or
 - i) F87V L188K A74G R47F V26T;
15. (currently amended) A method as claimed in ~~claim 13~~ claim 12, wherein the ~~hydroxylatable carboxylic acid is a C₄₂-C₃₀ monocarboxylic acid or a derivative thereof~~ and the monooxygenase (SEQ ID NO: 2) employed is a mutant selected from amongst the single mutants F87A, F87V, V26T, S72G, A74G and M354T, and the multiple mutants
- F87A L188K A74G R47F;
- F87V L188K A74G R47F;
- F87A L188K A74G R47F V26T; or
- F87V L 188K A74G R47F V26T.
16. (previously presented) A method a claimed in claim 12, wherein the reaction is carried out in the presence of an electron donor or a reduction equivalent.
17. (previously presented) A method as claimed in claim 16, wherein the electron donor or the reduction equivalent is selected from amongst NADH, NADPH and zn/CO(III) sepulchrates.